

The role of the roots in the life strategy of *Colchicum autumnale*

Lenka FRANKOVÁ*¹, Katarína CIBÍROVÁ², Karoly BÓKA³, Otilia GAŠPARÍKOVÁ¹
& Mikuláš PŠENÁK²

¹*Institute of Botany, Slovak Academy of Science, Dúbravská cesta 14, SK-84523 Bratislava, Slovakia; Tel.: ++421-2-59426119; *e-mail: farmlienka@hotmail.com*

²*Comenius University, Faculty of Pharmacy, Department of Cell and Molecular Biology of Drugs, Kalinčiakova 8, SK-83232 Bratislava, Slovakia; tel.: ++421-2-50259279*

³*Department of Plant Anatomy, ELTE, Pázmány Péter Setány 1/C, H-1117 Budapest, Hungary*

FRANKOVÁ, L., CIBÍROVÁ, K., BÓKA, K., GAŠPARÍKOVÁ, O. & PŠENÁK, M.,
The role of the roots in the life strategy of *Colchicum autumnale*. *Biologia*,
Bratislava, 59/Suppl. 13: 87–93, 2004; ISSN 0006-3088.

C. autumnale L. has an unusual life-cycle consisting of two growth stages: the first – autumnal stage (September-December) followed by winter season and the second – photosynthetically active one (March-May) leading to senescence (June) and dormancy (Jul-August). Within this cycle the roots exist for determined period. The root system appears first at the end of September (in the middle of flowering) and remains active till May, when the programmed senescence of above-ground part and the final physical destruction of mother corm take place. Our findings showed that these adventitious roots grow out from the root disk of new daughter corm and besides the nutritional function they possess contractional one. In case of seedling, the thick roots were observed on the new cormlet. It seems that thick roots together with well developed protuberance help the cormlet to descent gradually into the soil. Although the root system of both, young and 10–15-year-old (clonal) plants persists for seven months, the major role within the life-strategy of *C. autumnale* has the mother corm (warehousing and providing of storages) with protuberance (penetration) and two buds for future generation (continuity and vegetative reproduction). The results of the structural and biochemical analyses indicate that the first autumnal stage is accompanied by rapid starch reutilization from the mother corm, supporting new corm and shoot development, flowering and formation of future above-ground part under the soil surface. The storage reserves of the mother corm showed to be the only source of reduced carbon and nitrogen for all those events. The rest of the starch content was completely reallocated from mother to daughter corm till the end of the second, spring stage. In this paper the content of storage polysaccharides and changes in amylolytic activities were determined. The results are discussed in context of the life strategy of this perennial geophyte.

Key words: amylolytic process, corm, geophyte, protuberance, starch, thick roots.

Introduction

Autumn crocus, alias naked lady, meadow saffron, wonder bulb etc., has been used as approved medical plant for more than 3000 years although it is poisonous. This plant has three specific features: 1) the ability to produce colchicinoids, therapeutically active alkaloids, 2) the unusual life cycle, 3) the geophytic life style (FRANKOVA et al., 2003–2004). Generally geophytes are plants, that survive by subterranean storage organ with renewal buds (RAUNKIAER, 1934) ensuring continuity of species. They can be divided into two groups – synanthous and hysternanthous one (DAFNI et al., 1981). The leaves of synanthous geophytes coexist with flowers in the identical stage of the life-cycle. In case of hysternanthous plants flowers occur in the first and leaves in another, the second developmental stage. Autumn crocus belongs to group of hysternanthous geophytes and is one of the most common species of *Colchicum* genus in Slovakia and Central Europe. This plant is well characterised at the morphological level (for description see JAEHN et al., 1985; JAEHN & ROUX, 1986), but the knowledge concerning of its physiological, structural and biochemical characteristics is still missing. To get closer into the life strategy of this plant species we paid attention to the detailed characterisation of life-cycle followed by biochemical analyses.

Material and methods

Plant material

Colchicum autumnale L. comes from a natural population (Malacky, Slovak Republic – 48°26'N, 17°02'E). The plants were collected in the middle of each month.

Microscopic analyses

Fresh and fixed (40% ethanol) plant organs were used for light and stereomicroscopic analyses.

Starch, fructans and free sugar content

For starch extraction and determination perchloric acid method was used (ROSE et al., 1991). Low and high molecular fructans were extracted according to MARSCHALL et al. (1998), their separation and detection was assayed as described SAVITCH et al. (2000). Free sugars were extracted with 80% ethanol and separated by paper chromatography (FRANKOVA et al., 2003–2004).

Enzyme preparation

1 g of acetone-dried material was extracted with 0.1 M Na-phosphate buffer, pH 7.0 in presence of 1 g Polyclar AT. After extraction (30 min continual steering) and

centrifugation (3000 g, 15 min, 4 °C), the sediment obtained was re-extracted (two times) as described. Proteins from combined extracts were precipitated by ammonium sulphate (70% saturation). After centrifugation (7000 g, 45 min) the sediment was dissolved in 0.1 M Na-phosphate buffer, pH 7.0 and dialysed against 0.01 M buffer over night. After centrifugation (1000g, 1 min) the obtained supernatants were used as enzyme solutions. All operations were carried out at 4 °C.

Determination of amylolytic activities

The total amylolytic activity (TAA) was determined with 3,5-dinitrosalicylic acid reagent (BERNFELD, 1955) using potato soluble starch (*Sigma*) as substrate. The reaction mixture contained 0.4 mL of substrate solution (8 mg of starch) in 0.1 M Na-phosphate buffer pH 7.5. The reaction was initiated by adding of 0.1 mL of suitably diluted enzyme solution into the reaction mixture. After 20 min at 30 °C 0.2 mL of 3,5-dinitrosalicylic acid reagent was added and the mixture was kept in boiling water bath for 10 min. After cooling 2 mL of distilled water was added and the optical density was measured at 540 nm. The control sample contained temperature inactivated enzyme solution (100 °C, 10 min, centrifugation). For calibration curve maltose was used as standard (range 50–250 µg). The reduction power was expressed as amount of units with reducing end released from α-glucan substrates and was defined as a total amylolytic activity (TAA).

Results and discussion

The annual bioprogram of autumn crocus consists of two growth-developmental stages: The first, autumnal one followed by winter season and the second one leading to senescence and dormancy. Central role in the developmental program plays the corm with its storage reserves, what is a typical feature of hysternanthous geophytes. The autumnal stage starts after summer dormancy period, i.e. at the end of August. A new corm and shoot are differentiated from a bud located on the protuberance of mother corm (Figs 1A, B). This shoot is in flower during September, but it still doesn't contain any roots (Fig. 1C). The root system appears first in the middle of October and remains active for next 7 months (Fig. 1D). These adventitious roots grow out of the root disk of the daughter corm (Fig. 7) and besides the nutritional function they possess the contractional one. So after flowering the development of the roots and future above-ground part takes place in the soil. At the end of November the new shoot consists of corm (equipped with already developed roots), four leaves, stem and fruits with non mature seeds (Figs 1D, 2A–D). Microscopic analyses showed the presence of two indetermined meristems on the new daughter corm (Fig. 2B). These

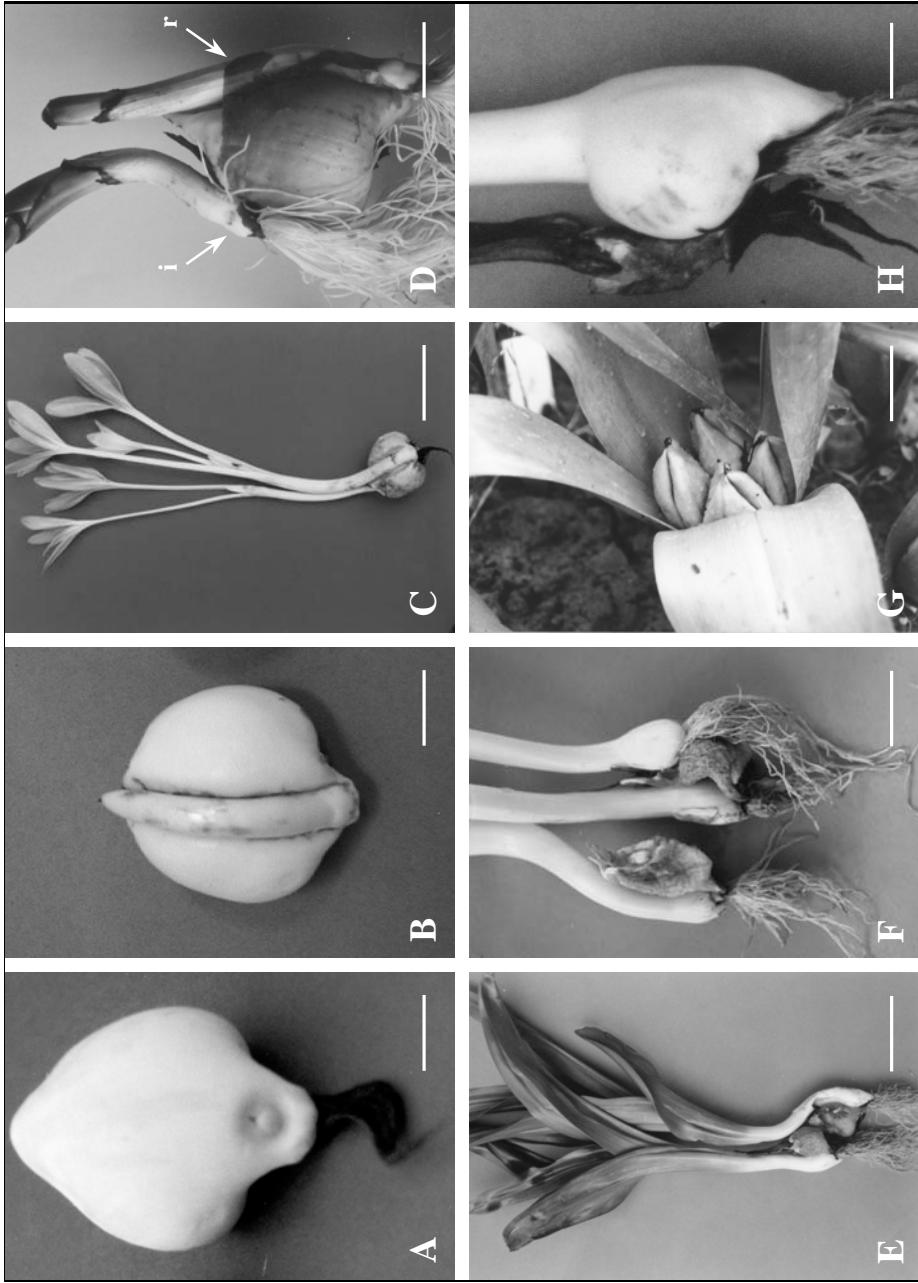


Fig. 1. The individual developmental stages of *C. autumnale* within its life-cycle: A – Mother corm with bud for future generation (July, bar 1 cm). B – Formation of the new shoot (the end of August, bar 1.2 cm). C – Flowering shoot without any roots (September, bar 4 cm); D – Mother corm with the new shoots (r – regular, i – irregular) including the future above-ground part and corm with roots (November, bar 1.5 cm). E – The rapidly growing leaves (March-April, bar 4 cm). F – The gradual growth of daughter corm and physical disappearing of mother corm (April-May, bar 1.5 cm). G – Capsules with ripening seeds (the end of May, bar 1.5 cm). H – Ripe new corm (the end of May, bar 1.2 cm).

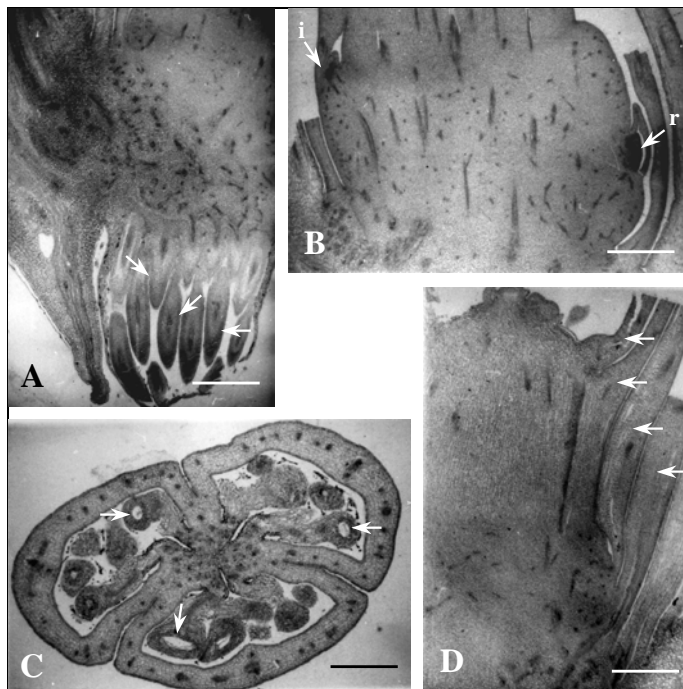


Fig. 2. Structural analyses of *C. autumnale* during autumnal stage: A – Roots growing out of the root disk (bar 1406 μm). B – The regular (r) and irregular (i) buds for future generations (bar 1125 μm). C – Young developing capsules with non-mature seeds (bar 563 μm). D – The future upper part of the plant (5 cm under the soil surface) including four leaves (bar 850 μm).

meristems remain intact for next ten months to form the bases of new generation within the coming life-cycle. The lower meristem (Fig. 2B) ensures the plant continuity by annual corm replacement and the second one is responsible for vegetative reproduction depending on the environmental conditions. So during this stage three generations of the plant coexist side by side – old mother corm, new daughter corm and two stored meristems.

It is very important to mention that the formation of whole future above-ground part starts in the soil without any light. And as till the end of September there are no roots and photosynthetically active leaves, the mother corm supplies all the building blocks (minerals, reduced carbon and mainly reduced nitrogen). In this relation the storage content and its degradation have been analysed. Our findings showed that starch was the main form of reduced carbon in corms of autumn crocus and no presence of fructans and mannans was observed. Fig. 3 indicates that starch represents about 50% of corm's dry mass. About 67% of its content is reutilised during autumnal stage (Fig. 3). At the same time the level of starch in the daughter corm is very low and constant. The decline in starch amount was accompanied by gradual increasing of TAA from August till December (Fig. 4). From enzymes involved in amyolytic process α -amylase, β -amylase and α -

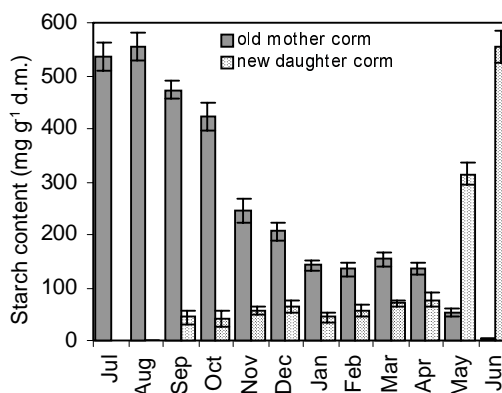


Fig. 3. The developmental profile of starch content in mother and daughter corms during the life-cycle of *C. autumnale*. (Means \pm SE, $n = 6$)

glucosidase have been identified (FRANKOVA et al., 2003–2004). The presence of pullulanase and starchphosphorylase was not observed. From free sugars sucrose, fructose and glucose were detected in both corms (Fig. 5). Maximal level of sucrose was observed during winter season, what may indicate its cryoprotective function. Our findings showed that starch degradation was key process of the first, autumnal stage. In the course of the second developmental stage, the rest of starch con-

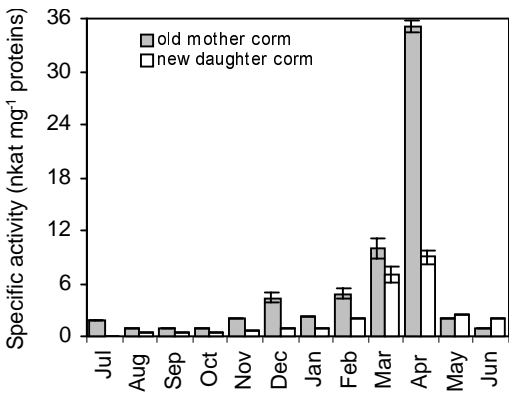


Fig. 4. The developmental changes in total amylolytic activity in mother and daughter corms during the life-cycle of *C. autumnale*. (Means \pm SE, $n = 6$)

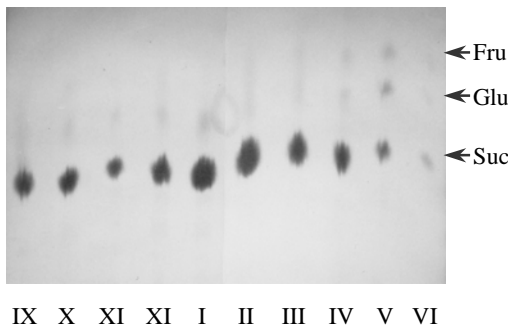


Fig. 5. The spectrum of free sugars in the mother corm during the life-cycle of *C. autumnale*: Fru – fructose, Glu – glucose, Suc – sucrose. Each line contained 25 μ g of fresh mass.

ment is completely reallocated from the mother into daughter corm. During this photosynthetically active period the individual organs of vegetative part are growing intensively (Fig. 1E), but the mother corm is gradually disappearing (Figs 1F, H). The above-ground part dies at the end of May and fruits are ripening (Fig. 1G). The starch formation in the ripening daughter corm is restricted to the short time period. It is of note, that final amount of starch in a ripe corm is equal to that in the mother corm at the beginning of the annual life-cycle (Fig. 3). The highest specific amylolytic activity was detected in both corms during March and April (Fig. 4). As the growth processes are very active at this time (Figs 1E, F), the rest of storages in the mother corm and the accessibility of photoassimilates can not saturate the intensity of carbon demand of the new corm. Therefore the

plant has to reach for already stored starch in new daughter corm. After programmed senescence of above-ground part the roots and mother corm die back also. What remains after each annual life-cycle are seeds and the ripe corm without roots (or with the rest of non-functional roots) equipped by two buds for future generation (Figs 1G, H). A corm at this form enters into summer dormancy and after that the life-cycle of clonal plant starts over again.

The annual corm replacement, ensuring continuity and perenniality of autumn crocus can be propagated generatively and vegetatively. The cormlets of seedlings of *C. autumnale* have special morphological characteristics. They contain a specific organ – protuberance, serving for its positioning in the soil. This structure together with contractile roots enables the gradual penetration of corm into the ground. Figures 6A-E show the individual generations and developmental stages within the life-cycle of 3-year-old plant. The position of previous mother corm is highly visible on Fig. 6E as well as the position of new daughter corm. The location of bud for future generation (regular corm) is indicated by arrow. Because every position is 1 cm away from previous one, each year the plant can get about 1 cm deeper into the ground. According to the location of mature plant (15–20 cm) in the soil it is evident, that it lasts about 15 years to reach the optimal depth of corm in the ground. In addition, the appearance of thick roots was observed on 4-year-old plant (Fig. 6F). Because the roots of young plant are very thin, these thick roots allow corm to overcome soil resistance. It is the first time when the two types of the roots were identified within life-cycle of *C. autumnale*. When plant becomes mature (it has the optimal depth and sufficient value of storage reserves) it can be reproduced also vegetatively. The irregular vegetative bud is positioned on the upper part of the mother corm (Figs 2B, 6G). The new corm formed from irregular bud is defined as irregular daughter corm, because it can be developed in any time of the spring or autumnal developmental stage of mature plant and doesn't ensure plant continuity. The new irregular corm serves for vegetative propagation and is positioned about 2–3 cm higher than the regular one (Figs 6B,I). So its protuberance together with contractile roots help the new plant to reach a deeper soil position, where the new lineage can be initiated (Fig. 6J).

The present results indicate the strong relationship between the life strategy of *C. autumnale* and the roots having predominantly nutritional and contractive functions. Although for

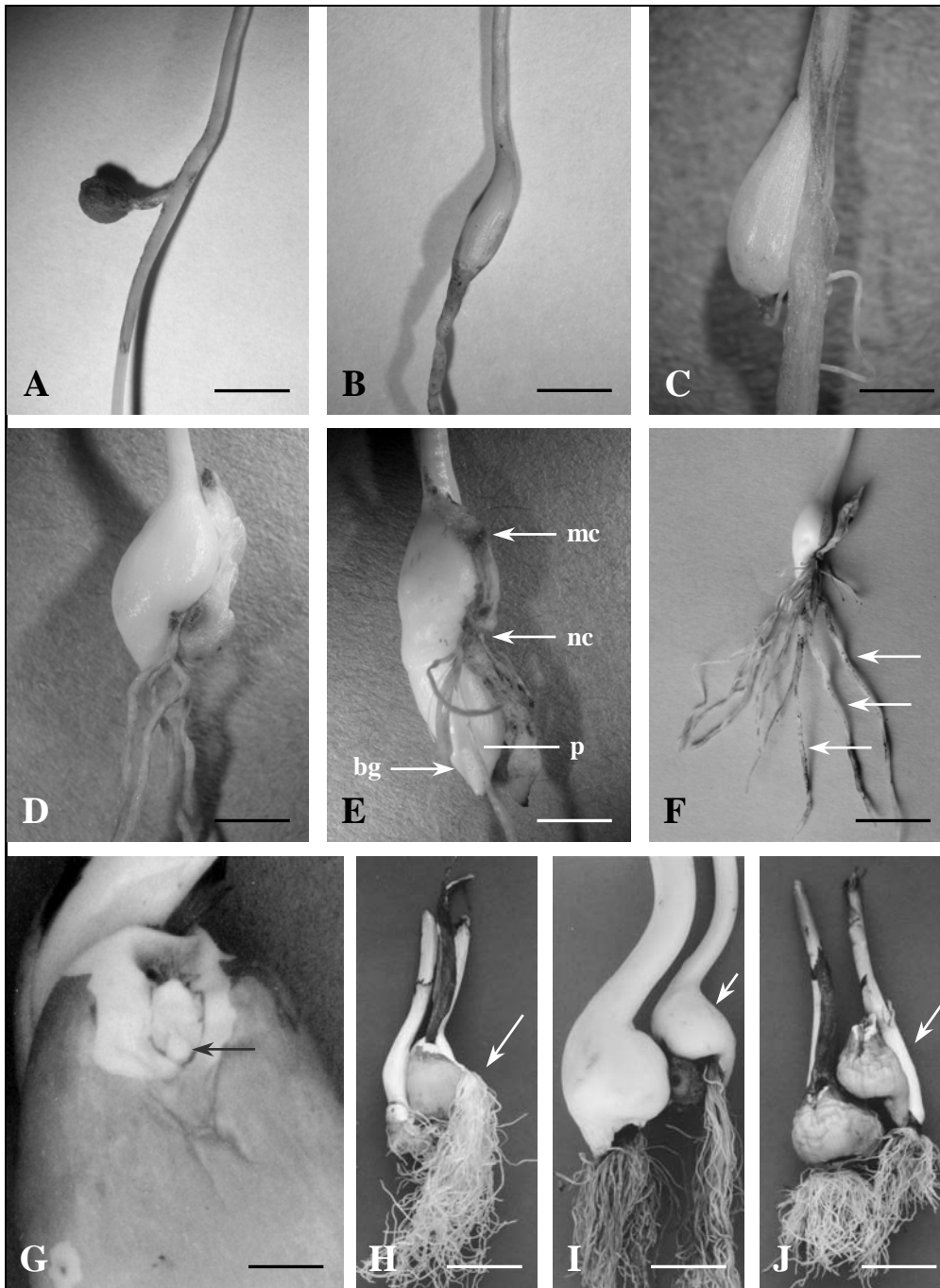


Fig. 6. The generative (A–F) and vegetative (G–J) reproduction of *C. autumnale*: A – Seedling, October (bar 3.75 mm). B – Seedling, April (bar 3.75 mm). C – Seedling, May (bar 1.5 mm). D – 2-year-old plantlet (bar 2.81 mm). E – 3-year-old plantlet (mc – the position of previous, old mother corm; nc – the position of new daughter corm; bg – the location of a bud for future generation, p – protuberance; bar 2.81 mm). F – 4-year-old plant with the thick roots (bar 1.2 cm). G – The initiation of independent lineage from irregular bud (bar 0.75 cm). H – The irregular corm at the end of autumn (bar 1.7 cm). I – The irregular corm at the end of spring (bar 2 cm). J – The independent lineage, next year (bar 1.7 cm).

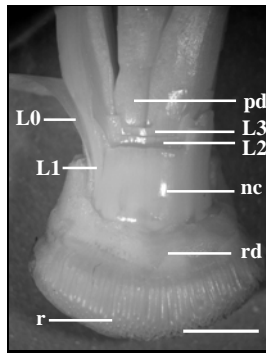


Fig. 7. Formation of the root system (October, bar 3.39 mm): r – Roots growing out of the root disk; rd – Root disk; nc – New daughter corm; L0 – The covering leaf (cataphyll); L1 – Part of the first leaf; L2 – Part of second leaf; L3 – Part of the third leaf; pd – Peduncle.

both young and clonal mature plants the root system exists for seven months, the subterranean storage organ has the major role within the life strategy, because it provides storages, perennality and vegetative propagation. To maintain perennality and vegetative propagation the appropriate level of starch must be guaranteed. Therefore the starch formation, degradation and its reallocation are of critical importance for *C. autumnale*. The knowledge of these relationships leads to better understanding of the life-strategy of *C. autumnale* as a representative of hysteroanthous geophytic life form.

Acknowledgements

This work was supported by Grant Agency of Slovak Ministry of Education, grant VEGA No. 1/1275/04.

References

- BERNFELD, P.: 1955. Amylases, α and β . *Methods Enzymol.* **1**: 149–158
- BÓKA, K., JAKAB, J. & KIRÁLY, I.: 2002. Comparison of the effect of differential fungal elicitors on *Rubia tinctorum* L. suspension culture. *Biol. Plant.* **45**: 281–290
- DAFNI, A., COHEN, D. & NOY-MEIR, I. 1981. Life-cycle variation in geophytes. *Ann. Missouri Bot. Gard.* **68**: 652–660
- FRANKOVÁ, L., KOMJÁTHYOVÁ, H., BÓKA, K. & PSENÁK, M. 2003–2004. Biochemical and physiological aspects of developmental cycle of *Colchicum autumnale* L. *Biol. Plant.* **47**: 509–516
- JAEHN, F., PFIRSCH, E. & ROUX, J. 1985. Zur Architektur des Jahresprosses der Herbstzeitlose (*Colchicum autumnale* L.). *Beitr. Biol. Pflanzen* **60**: 303–311
- JAEHN, F. & ROUX, J. 1986. Architecture et cycle annuel du colchique adulte (*Colchicum autumnale* L.). *Bull. Soc. bot. Fr.* **133**, Lettres bot.: 225–233
- MARSCHALL, M., PROCTOR, M. C. F. & SMIRNOFF, N. 1998. Carbohydrate composition and invertase activity of the leafy liverwort *Porella platyphylla*. *New Phytol.* **138**: 343–353
- RAUNKIAER, C. 1934. *The life forms of plants and statistical geography.* Oxford University Press, Oxford UK.
- SAVITCH, E. V., HARNEY, T. & HUNER, N. P. A. 2000. Sucrose metabolism in spring and winter wheat in response to high irradiance, cold stress and cold acclimatisation. *Physiol. Plant.* **108**: 270–278

Received Oct. 23, 2004

Accepted April 27, 2004